

Inventor(s): Elbaz et al. Application No: 09/762,194 Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

1/14

LOCUS

AT2 receptor C-terminal end

160 BP DS-DNA

ORGANISM BASES

Mouse 41 A

33 · C

36 G

50 T

Nucleic acids

1 TGTGTTAATC CCTTCCTGTA TTGTTTTGTT GGAAACCGCT TCCAACAGAA CGTCCGCAGT GTGTTTAGAG TTCCCATTAC TTGGCTCCAA GGCAAGAGAG AGACTATGTC TTGCAGAAAA

121 GGCAGTTCTC TTAGAGAAAT GGACACCTTT GTGTCTTAAA

#### Translation into amino acids

CVNPFLYCFV GNRFQQNVRS VFRVPITWLQ GKRETMSCRK GSSLREMDTFVS.

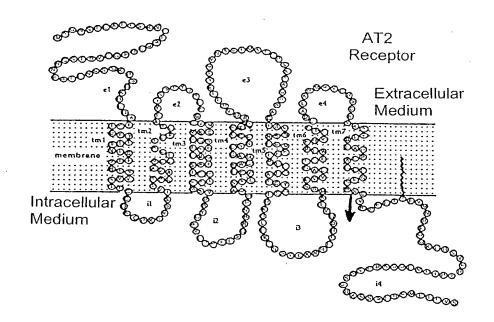


Figure 1



Inventor(s): Elbaz et al. Application No: 09/762,194 Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

2/14

Codon 147 .... AGT AAC AAA GGT CAA AGA CAG TTG ACT GTA TCG GAL4 DNA-binding domain Multiple Cloning Site

CCG GAA TTC CCG GGG ATC CGT CGA CCT... Sall BamHI Smal **EcoRI** 

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3/14

	GCTA	CTACCCCCCCCACGCACCCCCAATCTGGGTGGCCTGGCATTAGCATGTAAGCTTGTTTTTCTCTGGC											71						
TGTATCTCTTGGCCTGGAAGAACCCCGAGTTGCCAAGAGACACAGTATGTGATGGTCCCTGGAAAAG											TGCT	143							
	TCC	CCTGC	CGAAC	TTCI	CCCZ	\CTG0	CTTC	GAAC	GAC A	M ATG C		L TTG 1		P CCC A	K AA 1	F TC T	-	L TA	9 204
	S TCC	T ACC	I ATC		V GTC		L CTA	T ACC	A GCC	K AAA	G GGA	L CTG	L CTT	R CGA	N AAC	L CTC	R CGG	L CTT	27 258
	PCCT		G GGG	L CTC	R AGG	K AAA	N AAC	_	V GTC		F TTC	H CAC	T ACA	V GTT	E ·GAA	K AAG	G GGC	R AGG	45 312
	Q CAG	K AAG	N TAA	P CCC	R AGG	S AGC	L CTG	C TGC	I ATC	Q CAG	T ACC	Q CAG	T ACA	A GCT	P CCA	D GAT	V GTG	L CTG	63 366
	S TCC	S TCC	E GAG	R AGA	T ACG	L CTT	E GAG	L TTG	A GCC	Q CAA	Y TAC	K AAG	T ACA	K AAA	C TGT	E GAA	S AGC	Q CAA	81 420
	S AGT	G GGA	F TTC	I ATC	L CTG	H CAC	L CTC	R AGG		L CTT		S TCC	R CGT	G GGT	N AAC	N AAC	K AAG	F TTT	99 474
1	E GAA	A GCG	L CTG	T ACA	V GTT	V GTG	I ATC	Q CAG	H CAC	L CTC	L CTG	S TCT	E GAG	R CGG	E GAG	E GAA	A GCA	L CTG	117 528
	K AAG	Q CAA	H CAC	K AAA	T ACC	L CTC	S TCT	Q CAA	E GAA	L CTT	V GTC	_	L CTC	R CGG	G GGA	E GAG	L CTA	V GTT	135 582
	A GCT	A GCT	S TCA	S AGC	A GCC	C TGT	E GAG	K AAG	L CTA	E GAA	K AAG	A GCT	R AGG	A GCT	D GAC	L TTA	Q CAG	T ACA	153 636
	A GCG	Y TAT	Q CAA	E GAA	F TTT	V GTC	Q CAG	K AAA	L CTA	N AAC	Q CAG	Q CAG	H CAT	Q CAG	T ACA	D GAC	R CGG	T ACG	171 690
	E GAA	L CTG	E GAG	N AAC	R CGG	L CTG	K AAG	D GAC	L TTA	Y TAC	T ACC	A GCA	E GAG	C TGT	E GAG	K AAG	L CTT	Q CAG	189 744
	S AGC	I ATT	Y TAC	I ATT	E GAG	E GAĠ	A GCA	E GAA	K AAA	Y TAT	K AAA	T ACT	Q CAA	L CTG	Q CAA	E GAG	Q CAG	F TTT	207 798
9,	D GAC	N AAC	L TTA	N AAC	A GCC	A GCC	H CAT	E GAG	T ACC	T ACT	K AAG	L CTT	E GAG	I ATT	E GAA	A GCT	S AGC	H CAC	225 852
	s TCG	E GAG	K AAG	V GTG	E GAA	L TTG	L CTG	K AAG	K AAG	T ACC	Y TAT	E GAA	T ACC	S TCC	L CTT	S TCA	E GAA	I ATC	243 906
	K AAG	K AAG	S AGC	H CAT	E GAG	M ATG	E GAG	K AAG	K AAG	S TCA		E GAG	D GAT	·L CTG	L CTT	N AAT	E GAG	K AAG	261 960
3	Q CAG	E GAA	S TCG	L CTG	E GAG	K AAA	Q CAA	I ATC	N AAT	D GAT	L CTG	K AAG	S AGT	E GAA	N AAC	D GAT	A GCT	L TTA	279 1014
	N AAC	E GAA	R AGG	L TTG	K AAA	S TCA	E GAG	E GAG	Q CAA	K AAG	Q CAA	L CTG	S TCA	R AGA	E GAG	K AAG	A GCG	N AAT	297 1068
	S TCC	K AAA	N AAC	P CCT	Q CAG	V GTC	M ATG	Y TAT	L CTG	E GAG	Q CAA	E GAA	L CTA	E GAA	S AGC	L CTG	K AAG	A GCT	315 1122

Figure 3.1

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4/14

V GTG	L TTA	E GAG	I ATC			E GAG					_	D GAC	M ATG	K AAG	L CTA	M ATG	K AAG	333 1176
M ATG	E GAA	K AAG				N AAT						D GAC	K AAG	L CTG	K AAG	R CGA	F TTC	351 1230
Q CAG	Q CAG	E GAA	N AAC	E GAG		L TTA					D GAC	K AAA	H CAC	M ATG	A GCA	I ATT	S TCA	369 1284
R AGG	Q CAA	L CTT	S TCC	T	E GAG	Q CAG			L CTG		E GAG	S TCC	L CTT	E GAG	K AAG	E GAG	S TCA	387 1338
K AAG	V GTC	N AAC	K AAG			S TCC						L CTT	L CTG	W TGG	K AAA	L CTG	H CAC	405 1392
N AAC	G GGA	D GAC		-		P CCC						S TCC			I ATC	P CCT	F TTC	423 1446
Q CAG	S TCC	P CCC	R AGG	N AAT	S TCT	_	S TCC			S AGC		S AGC	I ATC	S TCA	CCC	R AGA	* TGA	440 1500
CGG	CTTC	rgaa(	CGCA	GGAG.	ACTC'	TCTG	AAGG	CACT	GAGG'	TGCG	CTTC	TGCA	GGAC'	TGAC	CCTC	TCAT	GGGA	1571
ACT	CGAG'	TTGC'	rgcg'	TTAG	CTCT	CTGG.	ААТА'	rccc	CAGG.	ATAT	CGGG	AGAG	CAGC	CGCC.	AACC	GTAT	CAGC	1642
TACGTACGAATAGAGAGCTCCAATAGAAGACTTTTAACTTGGTCCAAAAAGCCTCCTCCAAAAACAGATTTC											1713							
GGAACTGAAGTGGACATAGTTGCACAAAGCACTTACGGAACGAGGGAACCTTGTTCTTTGCCTTCCTT											1784							
CTAAGCATAGGCTTTCCAG											1803							

### Figure 3.2

Inventor(s): Elbaz et al. Application No: 09/762,194 Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

5/14

72

144 cctctgtggtggaatgacatttgctgtgtaggcatctttcctctgactgtatttcttggccttgaagagtac 216 tgagtttaaaaagacagtatgtgacagtccatggaaattgcctcttctgtgaaatctcgccacctgctccga 288 agac ATG TTG TCT CCC AAA TTC TCC TTA TCC ACC ATT CAC ATA CGA CTG ACG 343 17 F S L s T Ι Н Ι R L L S P K GCC AAA GGA TTG CTT CGA AAC CTT CGA CTT CCT TCA GGG TTT AGG AGA AGC ACT 397 S Т 35 L R P S G F R L R Ν L G 451 GTT GTT TTC CAC ACA GTT GAA AAG AGC AGG CAA AAG AAT CCT CGA AGC TTA TGT S R K N ₽ R S L C 53 F Η E K Q V 505 GAT GCG CTG CCC CCT GAG AAA ACA CTT GAA TTG ATC CAG CCA CAG ACA GCT CCC 71 ₽ D Α L P Р E K Т L E L Ι 0 Т ATC CTG CAG CTC AAG 559 CAA AGT GGA TTT ACG CAA TAT AAA ACA AAA TGT GAA AAC S F Ι L Q L K 89 N Q G Y K T K C E ACA GTT GCC TGT GGT AAT ACC AAG TTTGAG GCA TTG GTG ATT CAG 613 CAG CTT CTTI 107 N Т K E Α L Т v V Q L Α  $\mathbf{C}$ G Q L 667 AAA ACC CTA TCT CAA CAC CTG CTG TCT GAG CGG GAG GAA GCA CTG AAA CAA CAC 125 K T L S Q Η S Ε R E E 0 L 721 / GAA GTC ACT GCT TCA ACC ACC TGTGAG AAA GTT CTC CGG GGA GAG CTA CTT AAC 143 E K G Ε Ĺ V Т A S Т т C E N L 775 ACA GTG TAT GAA GCA TTC GTC CAG CAG AAT GAG TTA CAA TTAGAA AAA GCC AGG E F 161 Т Y A E Κ Α R ·N E L 0 L GAG TTT 829 TAC ACC AGG CGA GAG AATCGG CTTAAA CAG GCT GAA AAA ACA GAA CAC 179 F Y Т R T E R E K E E K Q ACT TAC ATT GAA GAA GCA GAG AAG TAC AAA ATG 883 GAG TATGAA AAG CTT CGG GAC 197 ĸ M D Т Y Т E F Α F. K Y Ε R 937 TAA CAT ACC TCT AAG TTG GAA CAA TTG CAA GAG CAG TTTGAC AAC TTA GCG GAA 215  $\mathbf{T}$ S K L Ε Α Н Ε Q L 0 Ε 0 F D N L N GCC TAT GAA GCC 991 CTT TTG CTA AAG AAG GAG GAA AAA TTA GAA GCT AGC CAC TCA K K Ē Α 233 E L L E K L Е A S Η S Ι 1045 GAA ATA GAA AAG AAA TCG CTT GAA GAT GAA ATT AAG AAA GGC CAT CTT TCA TCC 251 G Η E K S L E D K K S L S Ε T 1099 CTG AAG AGT TCG CTA GAG AAG CAA ATC AAT GAT CTT TCT GAG AAG CAG GAA ATT 269 S E S L E K 0 Ι Ν D L K S E К Q L AAAAGA GCA 1153 AGA AAA TTG AAA TCA GAA GAA CAA AAT GAT GCT TTA TAA GAA GAA 287 K R R Α Ε K L K S Е E Q Ε N D Α Ν L CTA CAG GAG TTA 1207 CCT CAG ATC ATG TAT GAA **AGA** GAA AAA GCA AAT TTG AAA AATĒ L 305 Ε Q K N Ρ Q I М Y L R E K Α

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6/14

GAA AGC CTG AAA GCT GTG TTA GAG ATC AAG AAT GAG AAA CTG CAT CAA CAG GAC 1261 323 E Ι K Ν Κ ATC AAG TTA ATG AAA ATG GAG AAA CTG GTG GAC AAC AAC ACA GCA TTG GTT GAC 1315 341 Ε K L V D Ν N Μ AAA TTG AAG CGT TTC CAG CAG GAG AAT GAA GAA TTG AAA GCT CGG ATG GAC AAG 1369 359 Ε Ν Ε Ε ĸ R М D CAC ATG GCA ATC TCA AGG CAG CTT TCC ACG GAG CAG GCT GTT CTG CAA GAG TCG 1423 377 S Т Ε Α V R CTG GAG AAG GAG TCG AAA GTC AAC AAG CGA CTC TCT ATG GAA AAC GAG GAG CTT 1477 395 E E Ν K V N K R L S M CTG TGG AAA CTG CAC AAT GGG GAC CTG TGT AGC CCC AAG AGA TCC CCC ACA TCC 1531 413  $\mathbf{C}$ S K N G D L Н TCC GCC ATC CCT TTG CAG TCA CCA AGG AAT TCG GGC TCC TTC CCT AGC CCC AGC 1585 431 S G S N ₽ R I L 0 S ATT TCA CCC AGA TGA cacgtccccaaagtccacagactctctgaaagcattttgatgcaggtctgc 1651 aggactgaccccaaggaggaacgtgggcacaagaggtatatcagcacacgtgtgatcaccgtaggtaactgg 1723 agcgtcaccaccggcggaatcgagcttctgagactggaagtctggaggaagacttttgcctccgtccaaaag 1795 attcctccaaaaaaagatttaaaaaaagatttcggcatcgacacggacgttgttgcacaaagcacttaaaga 1867 acgagagcatcttgttcattgcctttttcacctaagcataaggggaaaaactctcagggccctattaagatt 1939 tataacctttgtaatgttcttcaccacagacaccttcttgtgagttttcagtctgactgtgggggtgggggg 2011 tgtgaatgaaatggatgtcacagagtgtcatgtgtctgatgcagcctcctctgctgtgtattaaatgtcaaa 2083 atagaagaaaaagcaatagttgcttgaattatgatcatctaccaccaactctgctcagccctgtaacagggt 2227 agggagagggtataacaggaagagctttgacttgtccctgtctatacattctctgtatcttttggggggtaac 2299 tgtgagcctaacactatcctgtaattcattttctcaggctatgtgtaaatgtagaaccctaatttttctata 2443 aaaaaacaaactaactaactgtgtaaagaaagaaaaagggaagtaccaatgggtttttccaccttatttta 2515 cetttgatetaccettgeagatttaacetgtettetteecteceattatteteatttteettttacetttet 2587 atgattttccagagccccagagccagctcatcttccaggtgctgaaaccactttccaaataaactaaagcct 2731 ggatttgatattacaaattttgggaaatcttagaataaagaacgagaacaaggaagtcattggctagtataa 2803 ttaagaaaggtaggattcagtgcttaccgatgatgcagtacttgatagaagaaaacagtctgggaggatagc 2875 gctcatttttcagttaccctttaaggagtccctttgtctttgggaaagtagcagaatggtccgcttctttcc 2947 

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Notice of Allowance Dated July 15 2004
Atty Dkt No: 033339/208804



#### 7/14

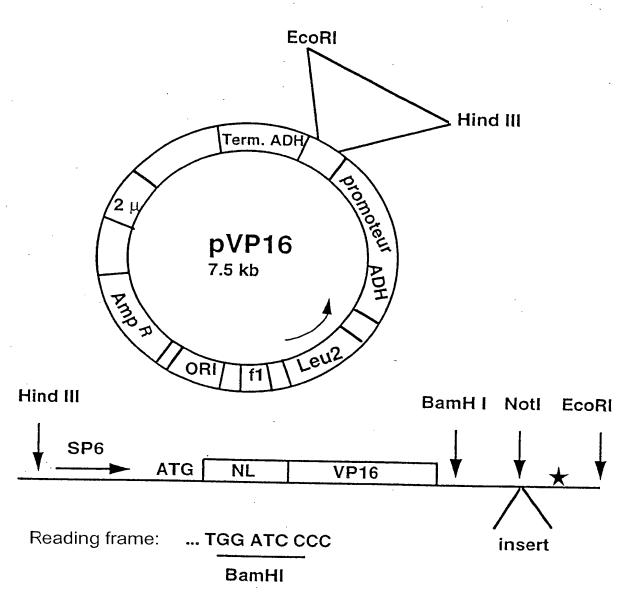
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gatgggaagtgtattttactctgatcaaataaataatgctggaatactcaaaaaaaa	3739
aaa	3742

### Figure 4.3



Inventor(s): Elbaz et al. Application No: 09/762,194 Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

8/14



★ Stop codons in three frames
pVP16 was constructed by Stan Hollenberg

Figure 5

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9/14

ATG	GAT	티	CGA	AAG	•
GGT	CGG	GGA TC	ATT	AGG	TAG
CAT	GGT	TGG	GGA	GAA	AAC
CAT	ATG	CGA	CAT	CCC	AAT
CAT	CAA	CTG TAC GAC GAT GAC GAT AAG GAT CGA TGG GGA TCC BamHI	GAG CTC GAG ATC TGC AGC TGG TAC CAT GGA ATT CGA	AGC TTG ATC CGG CTG CTA ACA AAG CCC GAA AGG AAG	AGC
CAT	CAG	AAG	TGG	ACA	CTG
CAT	GGA	GAT	AGC	CTA	CCG
CAT	GGT	GAC	TGC	CTG	CCA
TCT	ACT	GAT	ATC		CFG
GGT	ATG	GAC	GAG	ATC	TGG
೧գգ	AGC	TAC	CTC	TTG	AGT
98. Arg cgg ggr rcr car car car car car ggr arg	134 GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT			AGC	278 CTG AGT TGG CTG CCG CTG AGC AAT AAC TAG
98	134	170	206	242	270

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Notice of Allowance Dated July 15 2004
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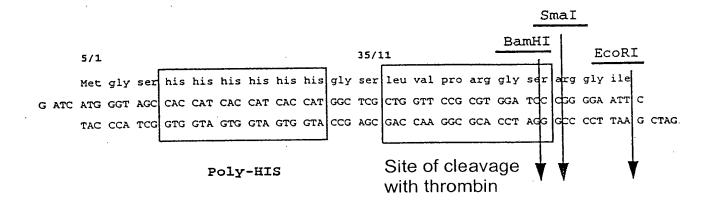
10/14

TAC CCA, GGC CTT GTC TTT GAC TAG AGA CTT CTT CTG GAC, CCT AGG CCT TAA GAT CT ATG GGT CCG GAA CAG AAA CTG ATC TCT GAA GAA GAC CTG'GGA TCC'GGA ATT CTA GA Met gly pro glu gln lys leu ile ser glu glu asp leu gly ser gly ile leu Ban Tag Myc

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Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

11/14



pBacPAKI-poly HIS -> Graphic Map

5526 b.p. AACGGCTCCGCC ... TCATTAATGCAG circular

PolyHIS insertion into pBackpack in BamHI (CACCAT) 3 1270-1287

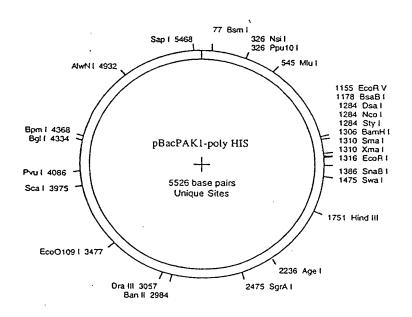


Figure 8

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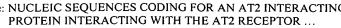
Atty Dkt No: 033339/208804

12/14

Tissues:

Skeletal Muscle: Liver Lung Placenta Pancreas Kidney Brain Heart Kb 9.5 7.5 4.4 2.4 1.3

Figure 9



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Inventor(s): Elbaz et al.
Application No: 09/762,194
Notice of Allowance Dated July 15 2004
Atty Dkt No: 0333339/208804



13/14

			← MBP-AT2	← GST-ATIP ← GSTalone		
.S:	MBP-AT1				! +	+
Supernatants:	MBP-AT2   MBPv   MBP-AT1				l +	+ 1
Sup	MBP-AT2	·	i 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		l +	+ I
	Z.	KDa	48 –	33	GST-ATIP	GSTalone
		Antibodies	anti-MBP	anti-GST	000000000000000000000000000000000000	

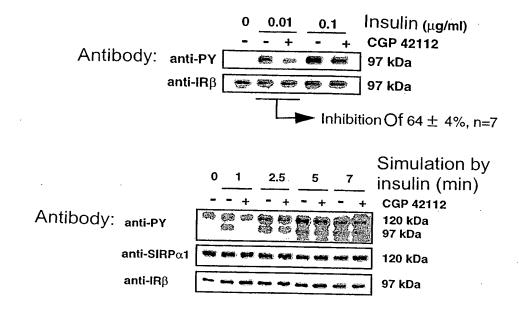


Inventor(s): Elbaz et al. Application No: 09/762,194 Notice of Allowance Dated July 15 2004 Atty Dkt No: 033339/208804

14/14

## CHO-hAT2

### Lectin column



## CHO-hAT2 et CHO-hAT2-ATIP

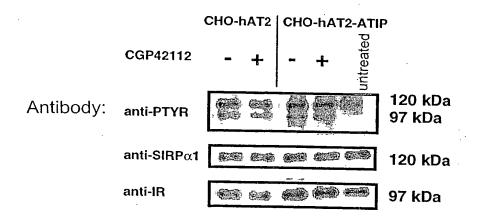


Figure 11